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Enzyme Immunoassay with Monoclonal Antibodies for the Detection of Rotavirus in Stool Specimens

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16. ABSTRACT

Rotavirus infections are generally recognized as a major problem in young children; however, they have also been associated with severe gastroenteritis in adults and neonates. Infections in neonates are usually asymptomatic, although the incidence of infection may be high. Adult infections also are often inapparent or mild, and thus, adults may be a reservoir of infection in pediatric wards. Diagnosis of infection is now routinely performed by enzyme immunoassay (EIA) in preference to the more cumbersome electron microscopy (EM). A commercial polyclonal EIA test, Rotazyme (Abbott Laboratories, North Chicago, Ill.), has been widely used for this purpose. The efficacy of this assay in adults has not been described, and reports of false-positive reactions in samples from neonates have resulted in recommendations that Rotazyme not be used with neonatal stool specimens. False-positive results in neonates have also been reported for other rotaviral EIA tests based on polyclonal sera. Because polyclonal sera may contain a number of cross-reacting components, the use of monoclonal antibodies should offer an advantage. Their sensitivity in immunoassays, however, may not be sufficient for a variety of reasons. Use of monoclonal antibodies as detector reagents for immunoassay of viral antigens has been limited, but they have been found to be equivalent or higher in sensitivity to polyclonal sera for detection of hepatitis B surface antigen in serum by RIA.

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Enzyme Immunoassay With Monoclonal Antibodies for the Detection of Rotavirus in Stool Specimens

Rotavirus infections are generally recognized as a major problem in young children; however, they have also been associated with severe gastroenteritis in adults and neonates [1]. Infections in neonates are usually asymptomatic, although the incidence of infection may be high. Adult infections also are often inapparent or mild, and thus, adults may be a reservoir of infection in pediatric wards.

Diagnosis of infection is now routinely performed by enzyme immunoassay (EIA) in preference to the more cumbersome electron microscopy (EM). A commercial polyclonal EIA test, Rotazyme® (Abbott Laboratories, North Chicago, Ill), has been widely used for this purpose [2–4]. The efficacy of this assay in adults has not been described, and reports of false-positive reactions in samples from neonates [5, 6] have resulted in recommendations that Rotazyme not be used with neonatal stool specimens [7]. False-positive results in neonates have also been reported for other rotaviral EIA tests based on polyclonal sera [8].

Because polyclonal sera may contain a number of cross-reacting components, the use of monoclonal antibodies should offer an advantage. Their sensitivity in immunoassays, however, may not be sufficient for a variety of reasons [9]. Use of monoclonal antibodies as detector reagents for immunoassay of viral antigens has been limited, but they have been found to be equivalent or higher in sensitivity to polyclonal sera for detection of hepatitis B surface antigen in serum by RIA [10]. Monoclonal and polyclonal antibodies to adenoviral hexon group antigen were found to be equivalent in sensitivity for identification by EIA of adenovirus in respiratory secretions and stools, but both tests gave <40% sensitivity when compared with isolation in cell culture [11]. An EIA test for rotaviral antigen with monoclonal antibody as the detector reagent has been described but did not include a clinical evaluation [12]. In this report, we describe a monoclonal antibody EIA and compare its sensitivity and specificity with that obtained by the polyclonal Rotazyme test and by EM on stool specimens from neonates, children, and adults.

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Please address requests for reprints to Dr. John E. Herrmann, Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, Massachusetts 01605.

Materials and Methods

Stool specimens. Seventy-four specimens from children (median age, 19 months) have been described [13]. All patients had gastroenteritis at the time stools were collected. Thirty-nine samples from adults involved in an outbreak of gastroenteritis were obtained from P. Echeverria (Bangkok, Thailand). Rotaviral serological data on acute and convalescent serum specimens from these adults were also available. Twenty-eight stool specimens from neonates (age, two months or less) were selected from samples previously tested by Rotazyme [5] and contained several samples that had been found to give false-positive results with that test. All of these samples, except one, were from patients who were asymptomatic at the time of sample collection. All stools were stored at −70 C. For assay, stools were prepared as 10% (wt/vol) suspensions in veal-infusion broth, except for stools from neonates, which had been diluted to 20% (wt/vol) in Rotazyme dilution buffer [5]. There was insufficient specimen volume to permit retesting of the neonatal stools by Rotazyme, as performed previously [5]. By further diluting the samples to a final 1:25 dilution, 11 of the stools that had given false-positive results by Rotazyme were able to be retested. Examination of stools for rotavirus by EM was done as described [14].

Antibodies. The monoclonal antibody used was prepared against murine rotavirus (EDIM) and reacted with the rotaviral group antigen (VP6). Details on the preparation and characterization of this antibody have been described previously [13]. Polyclonal antisera were prepared against purified simian SA-11 rotavirus in rabbits and goats, as we have reported [13].

Immunoassay. The wells of a 96-well polyvinyl chloride microtiter plate (Dynatech Laboratories, Alexandria, Va) were coated with 0.1 ml of rabbit antibody to SA-11, diluted in 0.01 *M* PBS (pH 7.0), for 4 hr at 37 C. The wells were postcoated overnight at 4 C with a solution of 1% (wt/vol) bovine serum albumin in PBS to help prevent nonspecific binding. For assay, plates were washed three times with PBS containing 0.05% Tween-20. Stool suspensions (0.05 ml/well) were added and incubated for 1 hr at 37 C. Plates were washed three times, and monoclonal antibody was added at 2 μg/ml (0.05 ml/well). After 1 hr at 37 C, plates were washed three times, 0.05 ml of peroxidase-labeled goat antibody to mouse IgG (Kirkegaard and Perry Laboratories, Gaithersburg, Md) at 1 μg/ml was added, and the plates were incubated for 1 hr at 37 C. The plates were then washed three times and 0.1 ml of peroxidase substrate (*o*-phenylenediamine + H_2O_2 in citrate-phosphate buffer; Abbott Laboratories) was added. After 15 min at room temperature, the reaction was stopped with 0.1 ml of 1 *N* H_2SO_4. The plates were read in a Dynatech plate reader at a wavelength of 490 nm. A positive test was one

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Table 1. Comparison of monoclonal and polyclonal enzyme immunoassays with electron microscopy for detection of rotavirus in stools.

Patient group	Percent sensitivity (no.)*		Percent specificity (no.)†	
	Monoclonal EIA	Polyclonal EIA	Monoclonal EIA	Polyclonal EIA
Neonates‡	100 (1/1)	100 (1/1)	100 (27/27)	37 (10/27)
Children	100 (29/29)	86.2 (25/29)	95.6 (43/45)§	95.6 (43/45)
Adults	100 (10/10)	50 (5/10)	96.5 (28/29)‖	89.7 (26/29)

* Results are percent sensitivity (no. of samples positive by EIA/no. of samples positive by EM) × 100.
† Results are percent specificity (no. of samples negative by EIA/no. of samples negative by EM) × 100.
‡ Samples from neonates were selected; see text.
§ The two EIA-positive, EM-negative samples were found positive by two confirmatory assays.
‖ One sample was positive by both EIA tests and by two confirmatory assays, but the stool specimen was negative by EM.

in which the A_{490} was ⩾0.15 above the negative control (pooled known negative stool suspension). Two confirmatory EIA tests were done, one by use of preimmune and immune sera for coating plates [9] and the other by a blocking test [13] with immune goat sera. Rotazyme was performed according to the manufacturer's directions.

Results

All 141 stool specimens were examined by EM, polyclonal antibody EIA, and monoclonal antibody EIA. Discrepant results between EIA and EM were resolved by two confirmatory EIA tests or by use of acute and convalescent sera in serological assays (adult samples). The standard by which the two EIA tests were measured was EM. The results are shown in table 1. The sensitivity of the monoclonal EIA was higher than that of the polyclonal EIA in both children and adults. The specificity of the immunoassays was the same in children. Both of the EIA-positive, EM-negative specimens were found positive by the two confirmatory assays cited above. All stool samples from adult patients who seroconverted for antibody to rotavirus were positive for rotavirus in stools by the monoclonal EIA and by EM, except for one that was negative by EM and positive by EIA. This sample was also positive by the confirmatory assays. Of 17 specimens from neonates that had been found to be false-positive by Rotazyme [5], none were positive by monoclonal EIA or EM. Of these 17 samples, 11 could be retested after further dilution, and six remained positive by Rotazyme. Thus, it is not likely that any of the false-positive results previously reported [5] were due to technical error or defective Rotazyme kits.

If data from confirmatory tests were included, there were no false-positive or false-negative results with the monoclonal EIA. Compared with EM only, there was a 97.4% agreement of the monoclonal EIA on all samples. The sensitivity of the assay was 100%, the specificity was 97.0%, and predictive values for a positive and negative test were 92.9% and 100%, respectively. The sensitivity of the polyclonal EIA on all samples was 77.5%. Excluding samples from neonates, the agreement of the polyclonal EIA with EM was 87.6%. The sensitivity was 76.9%, the specificity was 93.2%, and the predictive values for a positive and negative test were 85.7% and 88.5%, respectively.

Discussion

Compared with the polyclonal EIA (Rotazyme), the data we present for the monoclonal antibody EIA show that greater sensitivity, as well as specificity, can be achieved with monoclonal antibody as the detector reagent. The test eliminated false-positive reactions by the polyclonal EIA in stool specimens from neonates and was far more sensitive than the polyclonal test for detecting EM-positive samples from children and adults. The sensitivity of the monoclonal antibody EIA was 100%, and there were no false-positive specimens, as determined by two confirmatory assays plus seroconversion data for the samples from adults. The sensitivity of the polyclonal Rotazyme assay for samples from adults was especially low (50%); the sensitivity in children (86.2%) was approximately that found recently by others [4].

It is generally agreed that to achieve maximum specificity, monoclonal antibodies are preferable to polyclonal antibodies for use in the detection of infectious agents. The potential problem for use of monoclonal antibodies is that sensitivity may be decreased, because these antibodies react with only one epitope of a given antigen. Thus, if there is a limited quantity of antigen containing a number of different epitopes, the sensitivity for direct detection of antigen in clinical samples could be decreased. This problem can be diminished by using the monoclonal antibody as a coating (capture) antibody and polyclonal serum as the detector, as we reported for a rotaviral RIA [13]. The advantage of using the monoclonal antibody for capture is that it may pick up a significant amount of an antigen with several different epitopes, even if only one

reacts with a given antibody. The disadvantage is that if polyclonal antibody is used as detector in an immunoassay, it may react nonspecifically with antigens adsorbed to the solid phase. This may occur even if inhibitors, such as serum, serum fractions, or gelatin, are included in the diluents to block nonspecific reactions. For example, we found that in stools containing high titers of rotavirus there was sufficient reactivity to give a positive test with microtiter plates coated with preimmune sera (lacking antibody to rotavirus by RIA) even though there was two to three times greater reactivity with plates coated with immune sera (authors' unpublished data). Also, we have previously shown that enteroviruses adsorb readily to plastics in the presence of other proteins, whether coating antibody was present or not [15].

The high efficiency of the monoclonal antibody EIA not only improves the accuracy over currently available immunoassays in screening young children for rotaviral infection but now permits accurate screening for rotaviral infection in neonates and adults. This is important for infection control in hospital environments and will be useful in epidemiological studies. Our findings also have implications for the successful use of monoclonal antibodies in enzyme immunoassays of enhanced sensitivity and specificity for the direct detection of other infectious agents in clinical specimens.

John E. Herrmann, Neil R. Blacklow, Dorothy M. Perron, George Cukor, Peter J. Krause, Jeffrey S. Hyams, Helen J. Barrett, Pearay L. Ogra

Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, Massachusetts; the Department of Pediatrics, University of Connecticut School of Medicine, Farmington, and the Hartford Hospital, Hartford, Connecticut; the State University of New York at Buffalo, and the Division of Infectious Diseases, Children's Hospital, Buffalo, New York

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